REMARKS

Overview

Claims 1-8, 10-16, 19-27, and 30-33 are pending and under examination. Claims 1, 6, 19, 23, 30 and 33 have been amended. Support for the amendments can be found throughout the specification and the claims as filed. Accordingly, these amendments do not raise an issue of new matter and entry thereof is respectfully requested. The Office Action mailed December 27, 2007, has been reviewed, and Applicants respectfully traverse all rejections for the reasons that follow.

Rejection Under 35 U.S.C. § 112, Second Paragraph

Claims 1-8, 10-16, 19-27 and 30-33 stand rejected under 35 U.S.C. § 112, second paragraph, as indefinite allegedly because the steps/instructions of the claims do not clearly relate to the preamble. The Office Action further indicates that it is unclear what relationship exists between the method steps. Applicants respectfully maintain that the claims are clear and definite. Nevertheless, to further prosecution, independent claims 1, 6, 19, 23, 30 and 33 have been amended to more clearly relate the body of the claim to the preamble and to more clearly provide antecedent basis for the terms recited in the steps of the claims. Accordingly, this ground of rejection has been rendered moot, and withdrawal of this rejection is respectfully requested.

Rejection Under 35 U.S.C. § 102

Claims 1-8, 12-15, 19, 22, 23, 25-27, 30, 31 and 33 stand rejected under 35 U.S.C. § 102(e) as allegedly anticipated by Palsson, U.S. publication 2002/0012939. The Examiner alleges that the "logic constraints" of independent claims 1, 6, 19, 23, 30 and 33 can include all definitions from the specification as filed as the claims do not limit the definition to a particular embodiment. The

Examiner further alleges that Palsson teaches using constraints which include at least regulatory constraints at paragraphs [0054] and [0059]. The Examiner additionally alleges that Palsson teaches that constraints may be applied to change a flux boundary to thereby produce an improved model, thus teaching applying constraints to produce an altered model.

Applicants point out that independent claims 1, 6, 19, 30 and 33 were amended in the previous response to explicitly recite "logic constraints comprising a regulation matrix." Thus, the logic constraints, as recited in the claims, include a regulation matrix, regardless of what additional embodiments disclosed in the specification may also be included. The issue is not whether Palsson describes constraints, rather, the issue is whether Palsson teaches logic constraints <u>comprising a regulation matrix</u>. Applicants respectfully maintain, for the reasons of record, that Palsson provides no teaching of a logic constraint comprising a regulation matrix, as claimed.

As discussed in the previous response filed February 2, 2007, the constraints described by Palsson are values imposed as a solution to a linear equation. These values are selected and applied by the user to limit particular fluxes or the objective function of the flux balance analysis. Therefore, Palsson does no more than describe flux balance analysis and setting constraints for biasing fluxes to a desired value in order to represent a maximum or minimum allowable flux or in order to make the value a requirement for satisfying the objective function. Accordingly, Palsson does not describe logic constraints comprising a regulation matrix, as claimed. In contrast, the specification teaches that flux balance models relying solely on stoichiometric balances and uptake rates lead to overly optimistic expectations and that the predictive capabilities can be improved by inclusion of logic constraints to ensure consistency between the flux balance analysis and the kinetic and regulatory loops of the network. For example, the specification teaches:

> Flux balance models, by relying solely on stoichiometric balances and uptake rates are guaranteed not to exclude any feasible flux distributions. <u>However, this versatility may lead to overly optimistic expectations</u> if the results are not interpreted properly. The flux distributions within the cell are ultimately uniquely determined by the regulatory mechanisms within the cell, the kinetic characteristics of cellular enzymes, and the expression of these enzymes. <u>Assuming cells operate in a stoichiometrically optimal fashion may yield metabolic flux distributions not available to the cell.</u> The present invention provides for multiple methods for tightening the predicted stoichiometric flux boundaries by FBA models. A first strategy involves attempting to ensure that flux changes identified through FBA are consistent, in a qualitative sense, with the kinetics and regulatory loops of the metabolic network. By uncovering unreachable domains within the stoichiometric flux boundaries the predictive capabilities are improved.

Application at page 10, para. 2 through page 11, line 3 (emphasis added).

The application further teaches that a regulatory matrix is imposed on the analysis using logic constraints that determine whether the optimal flux distributions predicted by flux balance analysis will be prohibited. For example, the application teaches:

> The key question addressed here is whether the optimal flux distributions predicted by the FBA models are reachable by the cell <u>or whether kinetic and/or regulatory boundaries will prohibit the system from reaching the stoichiometric boundaries</u> (see Figure 4).
>
> The key idea we propose to explore is to ensure, <u>by using logic relations</u>, that when in response to environmental changes, the metabolic network shifts from one steady-state to another, up or down changes in metabolite concentrations are consistent with up or down changes in reaction fluxes.

Id. at page 11, para. 1-2 (emphasis added).

The specification at page 11, paragraph 3, through the paragraph bridging pages 12-13 further teaches that logic constraints incorporated into a flux balance analysis framework requires a regulation matrix F to be established that describes the effects of a metabolite on a reaction for each metabolite *i* and each reaction *j*. Exemplary logic constrains incorporated into a flux balance model which maintain consistency with the kinetic and regulatory barriers are shown in Equations 1-3 (page 12). As described therein, these constraints yield the equations set

forth in the first paragraph of page 13. Such logic constraints are distinct from the values described by Palsson to limit fluxes in a flux balance analysis.

As discussed previously in the response filed February 2, 2007, Palsson neither describes the use of kinetic and regulatory boundaries in combination with flux balance analysis to determine whether they prohibit the stoichiometric boundaries nor does Palsson describe incorporating the claimed logic constraints comprising a regulation matrix. In contrast to Palsson, the specification teaches incorporating logic constraints into a flux balance analysis framework using a regulation matrix that describes the effect of metabolites on reactions (page 12, first paragraph). Palsson provides no teaching of a logic constraint comprising a regulation matrix, and therefore Palsson does not teach each limitation of the claim, as required for an anticipatory reference.

In the Office Action on page 6, it is asserted that Palsson teaches using constraints which include at least regulatory constraints, referring to paragraphs [0054] and [0059]. Paragraphs [0054] and [0059] of Palsson read as follows:

> [0054] Constraints are placed on the network to account for the availability of substrates for the growth of *E. coli.* In the initial deletion analysis, growth was simulated in an aerobic glucose minimal media culture. Therefore, the constraints are set to allow for the components included in the media to be taken up. The specific uptake rate can be included if the value is known, otherwise, an unlimited supply can be provided. The uptake rate of glucose and oxygen have been determined for *E. coli* (Neidhardt et. al., *Escherichia coli and Salmonella: Cellular and Molecular Biology*, Second Edition, ASM Press, Washington D.C., 1996. Therefore, these values are included in the analysis. The uptake rate for phosphate, sulfur, and nitrogen source is not precisely known, so constraints on the fluxes for the uptake of these important substrates is not included, and the metabolic network is allowed to take up any required amount of these substrates.
>
> [0059] For this example, the objective of maximization of biomass yield is utilized (as described above). The constraints on the system are also set accordingly (as described above). However, in this example, a change in the availability of a key substrate is leading to changes in the metabolic behavior. The change in the parameter is reflected as a change in the uptake flux. Therefore, the maximal allowable oxygen uptake rate is changed to generate this

data. The figure demonstrates how several fluxes in the metabolic network will change as the oxygen uptake flux is continuously decreased. Therefore, the constraints on the fluxes is identical to what is described in the previous section, however, the oxygen uptake rate is set to coincide with the point in the diagram.

Contrary to the assertion in the Office Action, no where in these paragraphs of Palsson is there any teaching of "using constraints which include at least regulatory constraints." As discussed above, Palsson provides no teaching of "regulatory constraints" or logic constraints comprising a regulatory matrix, as recited in the claims. Thus, Palsson neither teaches the logic constraints comprising a regulatory matrix nor does Palsson teach applying such logic constraints to produce an altered balance analysis model.

Furthermore, Palsson provides no teaching of applying constraints to the flux balance analysis model, wherein the constraints include qualitative kinetic information constraints, qualitative regulatory information constraints, differential DNA microarray experimental data constraints, or a combination thereof, as recited in claim 23. The Office Action on page 4 alleges that Palsson teaches the use of differential DNA microarray data, referring to paragraph 60. However, in contrast to the claimed method, Palsson provides no teaching of differential DNA microarray experimental data <u>as a constraint</u>. Paragraph [0060] reads as follows:

[0060] Corresponding experimental data sets are now becoming available. Using high-density oligonucleotide arrays the expression levels of nearly every gene in *Saccharomyces cerevisiae* can now be analyzed under various growth conditions. From these studies it was shown that nearly 90% of all yeast mRNAs are present in growth on rich and minimal media, while a large number of mRNAs were shown to be differentially expressed under these two conditions. Another recent article shows how the metabolic and genetic control of gene expression can be studied on a genomic scale using DNA microarray technology (Exploring the Metabolic and Genetic Control of Gene Expression on a Genomic Scale, *Science*, Vol. 278, Oct. 24, 1997. The temporal changes in genetic expression profiles that occur during the diauxic shift in *S. cerevisiae* were observed for every known expressed sequence tag (EST) in this genome. As shown above, FBA can be used to qualitatively simulate shifts in metabolic genotype expression patterns due to alterations in growth environments. <u>Thus, FBA can serve to complement current studies in metabolic gene expression, by providing a fundamental approach to analyze, interpret, and predict the data from such experiments</u>. [emphasis added]

At best, Palsson describes using expression data from arrays <u>to corroborate</u> the flux balance analysis (FBA) methods described by Palsson, not as a constraint on the FBA. Thus, Palsson provides no teaching of applying constraints to the flux balance analysis model, wherein the constraints include qualitative kinetic information constraints, qualitative regulatory information constraints, differential DNA microarray experimental data constraints, or a combination thereof, as recited in claim 23.

For the reasons of record and as discussed above, Applicants respectfully maintain that Palsson does not teach the claimed methods or system. Absent such a teaching, Palsson cannot anticipate the claims. Accordingly, this ground of rejection is moot, and Applicants respectfully request withdrawal of this rejection.

<u>Conclusion</u>

In light of the amendments and remarks herein, Applicants submit that the claims are now in condition for allowance and respectfully request a notice to this effect. The Examiner is invited to call the undersigned if there are any questions.

No fees or extensions of time are believed to be due in connection with this amendment; however, consider this a request for any extension inadvertently omitted, and charge any additional fees to Deposit Account No. 26-0084.

Reconsideration and allowance is respectfully requested.

Respectfully submitted,

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